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DETECTION OF AFLATOXINS RESIDUES IN CHICKEN GIBLETS

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SUMMARY

Sixty chicken giblets samples, (20 each of gizzard and liver and 10 each of heart and canned liver) were randomly collected from different supermarkets and poultry butcher shops in Cairo, and analyzed for aflatoxins residues. The incidence of total aflatoxins (B_1, B_2, G_1 & G_2) and aflatoxin M_1 in examined samples were 100%. The highest total aflatoxins and aflatoxin M_1 residues levels (8.890 and 0.424 ppb) were estimated in canned liver and liver samples respectively, while the lowest levels (0.425 and 0.216 ppb) were estimated in gizzard and heart samples respectively. The public health hazards of aflatoxins as well as suggestive control measures were discussed.

INTRODUCTION

One of the food products of non negligible concern in the Egyptian markets is the chicken giblets namely liver, heart and gizzard prepared and available for sale by the poultry butcher's in their own shops. It plays an important role in nutrition as a contributer of high quality protein, energy and high vitamin contents to a world that is often undernourished, however, it is a highly perishable food due to low price.

Contamination of food with mycotoxins has considerable worldwide significance in terms of public health. Some foods may not carry any visible evidence of mould growth, yet may still bear mycotoxins. Poultry may fit truism when being fed on a mouldy ration.

Mycotoxins comprise a structurally diverse family of fungal elaborating metabolites which can induce toxicity in humans and animals. At present over 200 different mycotoxins are known, however aflatoxins are considered to be the most dangerous which are produced by *Aspergillus flavus* and *Asp.parasiticus* (Wyllie and Morehouse, 1978; Sanchis et al., 1986 and Pitt, 1989).

Mycotoxins may reach the consumer by two different ways: (1) The direct route via ingestion of cereals, nuts or fruits and other plant commodities as well as meat which are spoiled by fungi, (2) An indirect exposure is known to occur when toxic products of mycotoxins persist in meat and other tissues as well as milk and egg from animal which have been exposed to mycotoxin contaminated feed- stuffs (Fink -Gremmels, 1992).

Mycotoxins are considered unavoidable contaminants in foods and feeds because agronomic technology has not yet been advanced to the stage at which preharvest infection of susceptible crops by fungi can be eliminated. Hence widespread and frequent monitoring surveys should be carried out. Several auothers reported that under experimental conditions mycotoxins and /or their metabolites can be traced in meat, edible tissues, milk and egg (Wood, 1992 & Gareis and Wolff, 2000).

Aflatoxins B_1, B_2, G_1 and G_2 are potent teratogenic, mutagenic, and carcinogenic mycotoxins, which are classified as Group 1 human carcinogens, whereas aflatoxin M_1 is classified as a Group 2 probable human carcinogens (FAO / WHO, 1995 and Park, 1995). Thus, it seems necessary to monitor the presence of mycotoxins in food to exclude a frequent exposure of consumers.

So the focus of this paper was to investigate the occurrence of aflatoxins in chicken giblets (gizzard, liver, heart and canned chicken liver) marketed in Cairo

MATERIAL AND METHODS

Aflatoxins analysis:

Using aflatoxins quantitative test kit (Veratox, Neogen Corporation) and Behring E 13115 Enzyme- Linked Immunosorbent Assay, Auto reader.

1. Preparation of sample extract:

1.1. fifty gm of samples were blended with 250 ml of 70% methanol / water solution for 2 minutes in a high- speed blender (Sterilimixer Lab. Pbi. International Milano Italy, 16500 giri/ min.).

1.2 The extract was filterated by pouring at least 5-15 ml through a Whatman #1 filter paper and the filtrate was collected as a sample.

2. Detection of aflatoxins residues:

2.1. Total Aflatoxins (B_1, B_2, G_1, & G_2):-

2.1.1. Remove 1 red-marked mixing well for each sample to be tested plus 4 red-marked wells for controls and placed in the well holder.

2.1.2. Remove an equal number of antibody-coated well. Mix each reagent by swirling the reagent bottle prior to use.

2.1.3. Place 100 mcl of conjugate in each mixing well.

2.1.4. Transfer 100 mcl of standard controls (0, 5, 15 and 50 ppb aflatoxins) and samples to the mixing wells.

2.1.5. Mix the wells by pipetting liquid up and down in the tips 3 times

2.1.6. Transfer 100 mcl from each mixing well to the corresponding antibody coated wells and mix by sliding the microwell holder back and forth rapidly on flat surface. Incubate 2 minutes at room temperature.

2.1.7. With a wash bottle or running stream of water, fill each antibody well with distilled water and dump them out. Repeat this step 5 times, then turn the wells upside down and tap out on a paper towel until the remaining water has been removed.

2.1.8. With new tips, pipette 100 mcl of substrate into the wells and mix. Incubate 3 minutes at room temperature.

2.1.9. Add 100 mcl red stop solution to each well and mix throughly

2.2 Aflatoxin M_1:-

2.2.1 Preparation of aflatoxin M1 standards: For each one standard bottle (0.0, 0.25, 0.5, 1.0 and 2.0 ppb) squeeze the content of blue marked tubes (water), then swirled to dissolve the standard and wait for at least one hour to run the examination.

2.2.2. Transfer 50 mcl of conjugate in each mixing wells.

2.2.3. Transfer 200 mcl each of standard and samples to the mixing wells, mix each well by pipetting up and down 3 times.

2.2.4. Transfer 100 mcl from each mixing well to the corresponding antibody coated well, mix by sliding the microwell holder back and forth rapidly on a flat surface. Incubate 30 minutes at room temperature.

2.2.5. After incubation, wash the wells by gentil running stream of water 10 times and turn the wells upside down and tap out on a paper towel until the remaining water has been removed.

2.2.6. Pipette 100 mcl of substrate solution into each well and mix then, incubate 10 minutes at room temperature.

2.2.7. Stop the reaction by using 100 mcl stopping solution into each well and mix thoroughly.

2.3. Estimation of aflatoxins residues:

2.3.1. The color of the resulting solutions for each of total aflatoxins and aflatoxin M_1 were observed. Blue color indicates negative samples, while red color indicates a strong positive.

2.3.2. Wipe bottom of microwells and read in a micro-well reader blanked on air using a 650 nm filter.

2.3.3. The concentration of aflatoxins in the samples are estimated from curves relating absorbency to the concentration of the aflatoxins standard {Fig. 1 and 2}. (Frohlich et al., 1997)

RESULTS AND DISCUSSION

The achieved results are recorded in tables 1, 2, 3 and 4.

It is evident from the results achieved in table (1) that the incidence of total aflatoxins (B_1, B_2, G_1 &G_2) and aflatoxin M_1 (produced by hydroxilation of the C-3 position of bis-furan ring of aflatoxin B_1) residues in chicken gizzard, liver, heart, and canned liver samples were 100%.

Nearly similar results have been recorded by Sova et al., (1982); Teleb and Fakhary, (1988) and Shaltout et al., (2001). while lower figures have been reported by Abd El- Khalik, (1985); Hegzi, (1988); Gab- Allah, (1995); Hammad (1995); El-Shewy et al., (1997); Shabana, (1999); Sayed et al., (2000) and El-Zeini et al., (2001). On the other hand, Reddy et al., (1982) and Salem, (1997) could not detect any level of aflatoxins from broiler liver and heart samples.

In the present study, the high incidence of aflatoxins residues in examined chicken giblets may be attributed to the very high incidence and concentration of aflatoxins up to 10.458 mg/kg in the poultry feed and its related feed stuffs in all over the world (Shotwell et al., 1973; Shotwell et al., 1978; Qutet, 1980; Abdel-Haleem, 1982; Soares and Rodrignez, 1989; Refai et al., 1990; Rizvi et al., 1990; El-far et al., 1993; Dutton and Kinsey, 1995 and Saleh, 1998), which perhaps due to climatic conditions specially when cereals crops were subjected to rain fall and /or high relative humidity during ripening, bad sanitary conditions during storage and exposure of the feed ingredients to fungal contamination due to temperature abuse, transportation and insect damage (Dickens, 1983 and Miller, 1995).

The statistical analytical results of total aflatoxins and aflatoxin M_1 residues (ppb) in chicken giblets were recorded in table (2). It ranged from 0.425 to 8.193 with a mean value 4.111 ± 0.527 and 0.235 to 0.419 with a mean value 0.301± 0.015, respectively for gizzard samples. While for liver samples the values were 0.453 to 7.045 with a mean value 3.778 ± 0.160 and 0.219 to 0.424 with a mean value 0.321 ± 0.017, respectively. Regarding heart samples, it was 2.045 to 6.190 with a mean value 4.609 ± 0.456 and 0.216 to 0.419 with a mean value 0.275 ± 0.019, respectively, Where as, for canned liver samples, it was, 2.973 to 8.890 with a mean value 6.750 ±0.619 and 0.232 to 0.341 with a mean value 0.297 ± 0.014, respectively.

From the aforementioned results, it is clear that the estimated aflatoxins residues in chicken giblets were nearly similar to those reported in the previous studies of Maikanov, (1986); Marvan et al., (1987) and Salem, (1997). Where as lower figures were established by Zakaria, (1985); Bukovjan et al. (1992), Gab- Allah, (1995) and El-banna et al., (2001). On the other hand higher results were reported by Teleb and Fakhary, (1988);

Sayed et al., (2000); El-Zeini et al., (2001) and Shaltout et al., (2001). However, Asim et al., (1990) reported much high values reaching up to 493 ppb in poultry livers. This is probably due to the fact that such data were carried out related to liver samples from morbid poultry.

From the previous data, it could be noticed that the total aflatoxins residues in canned liver were relatively higher than those determined in other organs. This may be attributed to spices and some other food additives which were investigated as the main important source of toxigenic moulds and mycotoxins (Scott and Kennedy, 1973; Flanniga and Hui, 1976 and Misra, 1981).

The over all data obtained during study indicated that the residue of aflatoxin M_1 in all examined samples was lower than the relevant total aflatoxins (B_1, B_2, G_1, G_2) in the same samples. In this respect, Klaassen, (1980) attributed the lower level of aflatoxins M_1 and M_2 in the liver and kidney samples than B_1 and B_2, to the higher polarity and increased water solubility of aflatoxins M1 and M_2.

The results obtained in table (3) showed the frequency distribution of the chicken giblets samples according to their amount of total aflatoxins (ppb).

Although that all chicken giblets samples do not exceed the permissible limit (10 ppb) established in Egypt, South Africa, Spain, Italy, Greece, Peru and Mauritius, in addition to USA (20 ppb), it was found that 11,11, 6 and 8 of gizzard, liver, heart and canned liver samples exceed the permissible limit (4 ppb) of UK, Germany and Denmark, but, 8, 7, 5 and 8 of gizzard, liver, heart and canned liver exceed the permissible limit (5 ppb) of Sweden, New Zealand, Norway, Bulgaria, Finland, Hong Kong and Cuba (FAO 1997).

Kinsman et al., (1994) stated that edible by-products particularly liver are employed in a large number of dishes or as common ingredients in baby foods and restoratives in many countries. Therefore, comparing the previous data to limit established in Nigeria and Argentina (B_1 0.0 ppb for infant foods), Honduras (B_1, B_2, G_1 & G_2 0.01 ppb for baby foods) and Austria (B_1, B_2, G_1, G_2 & M_1 0.02 ppb), it was found that the examined chicken giblet samples exceeded such limit. On the other hand 13, 13, 9 and 10 of gizzard, liver, heart and canned liver exceed the limit for baby foods (3ppb) established in Brazil and Czech Republic (FAO 1997).

Table (4) summarizes the frequency distribution of the chicken giblets samples according to their amount of aflatoxin M_1 (ppb) residues.

Comparing the achieved data to limits established in Russia (0.5 ppb for animal fats) and Czech Republic (5.0 ppb for all foods), it was found that all chicken giblets do not exceed the limits, while all

Table (1): Incidence of total aflatoxins (B_1,B_2,G_1, & G_2) and aflatoxin M_1 residues in the analyzed chicken giblets

Chickens giblets	No. of samples	Total Aflatoxins		Aflatoxins M_1	
		No.	%	No.	%
Gizzard	20	20	100	20	100
Liver	20	20	100	20	100
Heart	10	10	100	10	100
Canned Liver	10	10	100	10	100

Table (2): Statistical analytical results of total aflatoxins (B_1,B_2.G_1 & G_2)) and aflatoxin M_1 residues (ppb) in the analyzed chicken giblets.

Chicken giblets	Total Aflatoxins			Aflatoxins M_1		
	Min	Max	Mean ± SE*	Min	Max	Mean ± SE*
Gizzard	0.425	8.193	4.111 ± 0.527	0.235	0.419	0.301± 0.015
Liver	0.453	7.045	3.778 ± 0.160	0.219	0.424	0.321 ± 0.017
Heart	2.045	6.190	4.609 ± 0.456	0.216	0.419	0.275 ± 0.019
Canned Liver	2.973	8.890	6.750 ± 0.619	0.232	0.341	0.297 ± 0.014

* SE = Standard Error.

Table (3): Frequency distribution of analyzed chicken giblets samples contaminated by different levels of total aflatoxins (B_1,B_2,G_1 and G_2)ppb.

Chickens Giblets	0.001-0.500	0.501-1.000	1.001-1.500	1.501-2.000	2.001-2.500	2.501-3.000	3.001-3.500	3.501-4.000	4.001-4.500	4.501-5.000	5.001-5.500	5.501-6.000	6.001-6.500	6.501-7.000	7.001-7.500	7.501-8.000	8.001-8.500	8.501-9-000
Gizzard	2	-	-	3	1	1	2	-	2	1	2	1	2	-	1	1	1	-
Liver	1	2	1	2	-	-	1	1	2	3	2	1	1	2	1	-	-	-
Heart	-	-	-	-	1	-	2	1	-	1	1	2	2	-	-	-	-	-
Canned Liver	-	-	-	-	-	1	-	1	-	-	-	-	1	-	2	3	1	1

Table (4): Frequency distributiion of analyzed chicken giblets samples contaminated by different levels of aflatoxin M_1 (ppb).

Chicken giblets	Frequency groups					
	≤0.22	0.23 - 0.25	0.251 - 0.3	0.31 - 0.35	0.351 - 0.4	0.41 - 0.42
Gizzard	-	4	10	-	2	4
Liver	1	1	8	2	3	5
Heart	2	1	5	1	-	1
Canned Liver	-	2	3	5	-	-

Fig.1

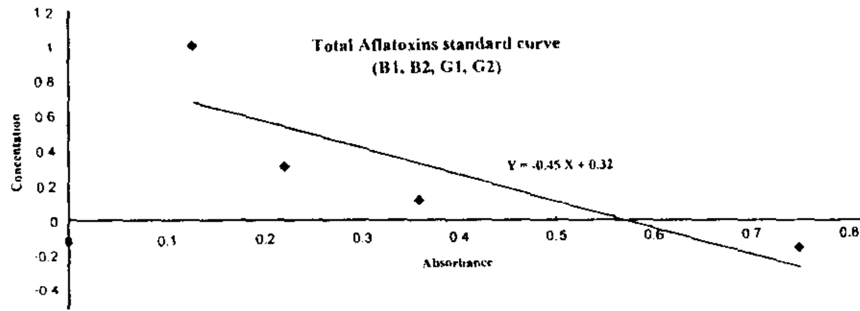
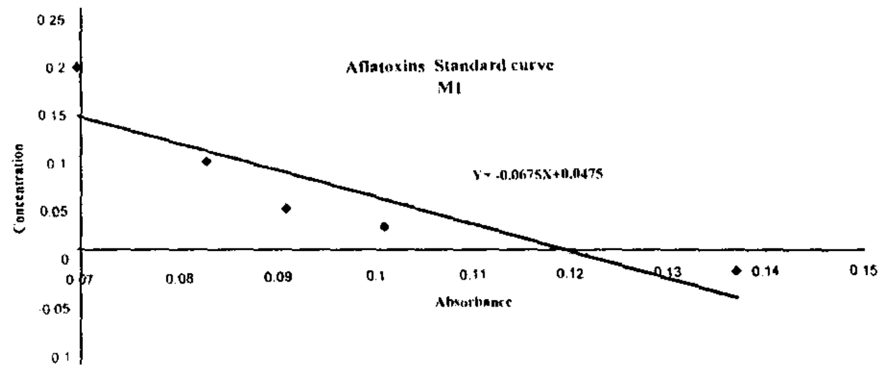


Fig.2



samples were more than the maximum tolerated levels of aflatoxin M_1 (0.0 ppb for milk and diary products) set by Egypt, (0.01 ppb) by Germany & Austria and (0.02 ppb) by Honduras and Switzerland (FAO 1997).

In conclusion, from the public health point of view aflatoxins may be regarded as a potent toxin, a carcinogen, a teratogen and mutagen (Ueno and Ueno, 1978 & Betina, 1989).

It is of importance to recognise that different food processing can't render the edible tissue safe as the reduction of the mycotoxins level is often insignificant, subsequently the risk from mycotoxins is still present.

So, it is essential to safe-guard the consumer from the possible mycotoxins residues in edible tissues through screening the poultry feed and its related ingredients before being fed to the broiler and laying hen's in order to have safe giblets free from aflatoxins.

The levels of accumulation were influenced predominantly by length of time free from contaminated feed prior to slaughter (Kryukov and Krupin, 1993). Micco et al., (1988) concluded that no detectable amounts of aflatoxins were found in any tissue after withdrawal periods of 14 and 33 days for male broilers and laying hens respectively.

The standard limits, national or international, must be fulfilled during the analysis of food and feed stuffs.

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